RAD DRAFT ENGINEERING REPORT (10/12/2018)

STANDARD REVIEW ENGINEERING REPORT [redacted]

MCAN: J18-0044 ENGINEER: Jackson/AFD (JAS Review)

SUBMITTER: Biosystems and Biomaterials Division
Material Measurement Laboratory
National Institute of Standards and Technology
100 Bureau Drive
Gaithersburg, MD 20899-8543

MICROORGANISMS:

Recipient/host (p. 2):

The recipient microorganism is *Saccharomyces cerevisiae* BY4739 (MATalpha leu2Δ0 lys2Δ0 ura3Δ0) derived from *S. cerevisiae* 288C.

Donors (p. 4-5):

- URA3 gene from *M. jannaschii* DSM 2661 for selection of transformed cells; thus, the subject microorganism can grow in both typical yeast broth (YPD) or in yeast synthetic defined (SD) broth without uracil.
- DNA sequence (ERCC-00095) from *Methanocaldococcus jannaschii* to give specificity to the strain (detection marker).

GEM: J18-0044 is referred to as *Saccharomyces cerevisiae* NE095 (NIST ERCC 00095) (p. 1).

PV (CFU/yr) (p. 23):

The submission states that production will be in a single batch of approximately 4L, with an expected cell concentration of 5 x 10^7 CFU/ml, and that this production is expected to provide a 5-year supply of the material. The plan for NIST RM 8230 is to produce approximately 200 units each containing 12 vials, with a target cell number of approximately 5 x 10_7 total cells/vial. The submission states that subsequent manufacture within the first three years is not expected. If another batch is needed within that time frame (likely in the second or third year), it

is anticipated that the second batch might be slightly larger, such as within three-fold of the original batch in terms of volume and amount of culture.

Original Batch (Year 1):

$PV = (5.0 \times 10^{7}$ CFU/mL)(4 L)(1000 mL/L)
$PV = 2.0 \times 10^{11}$ CFU/yr (1 batch/yr)

Potential 2nd Batch (Year 3):

$PV = (5.0 \times 10^{7}$ CFU/mL)(12 L)(1000 mL/L)
$PV = 6.0 \times 10^{11}$ CFU/yr (1 batch/yr)

USE:

The majority (> 70 %) of the use is expected to be for training and exercises relevant to biological detection. The remaining ≈ 30 % of use cases are expected to focus on validation of microbial enumeration technologies. (p. 23-24).

SUMMARY:

The specific insertions/deletions (and corresponding effects) associated with the GEM are discussed in detail on pages 3-22 of the submission.

Submission states that all manufacturing in the next 3 years will occur at a toll site (likely Microbiologics, Inc. in St. Cloud, MN) (p. 23 and technical contact). Seed and main fermentors are used to grow the MCAN. Upon completion of the fermentation, the vials containing the GEM are sent to other sites for the downstream training and biological detections uses. The broth is inactivated through autoclaving or exposure to fresh sodium hypochlorite solution (10% by volume) for 20 min. In addition, the submitter states that the lyophilization has a 90% inactivation efficiency (p. 23).

NOTES AND KEY ASSUMPTIONS

The 1997 Biotechnology Generic Scenario was referenced in this IRER. The technical contact was called – see contact report.

The submitter submitted a past TERA case on the same recipient organism, which was used on metal detection coupons, open air release, and laboratory studies (R15-0003). Submission indicates that this MCAN is used for biosafety training purposes or used in laboratory instruments. For conservative releases/exposures, RAD assumes 100% of the MCAN is used for training. The technical contact was called (see contact report).

Sporulation

The technical contact stated that the GEM does not produce spores (see contact report). Therefore, RAD did not assess spore releases.

INITIAL REVIEW ENGINEERING REPORT CBI: Yes

MCAN: J18-0044

MANUFACTURING/PROCESSING: Lyophilization of MCAN

Sites/Locations:

Manufacturing site not controlled by submitter and not yet identified (p. 1 and technical contact)

Days/yr: 2 days/yr

Basis: The technical contact estimates that the process would only happen once a year and take approximately 2 days (see contact report)

PROCESS DESCRIPTION:

The MCAN strain is sent to toll manufacturing site (likely Microbiologics, Inc. in St. Cloud, MN, per technical contact) for the lyophilization process. The exact processing operation will depend on the contract awardee for the material production. However, the expected processing steps would include culturing, resuspending in lyophilization matrix, aliquoting into individual samples, lyophilizing (or freeze-drying yeast containing the GEM), characterizing via colony growth on agar plates, and packaging into vials (p. 23-24).

The technical contact has awarded Microbiologics, Inc. similar contracts in the past (R15-0003; and the process operation for the same GEM was the following: "custom lyophilization into individual pellets of cells encased in a proprietary lyoprotectant matrix…This process produces 100g of wet mass, which is then dried to get about 1/3 volume. The dry yeast is then milled down to powder with particle sizes of 100 microns. We would also like to add silica fluidizer to help flow before making into pellets… Preliminary measurements suggest ~10^6 to ~10^7 CFU per pellet and ~10^7 to ~10^8 total cells per pellet." (per same submitter analog past case R15-0003).

RAD produced a flow diagram to show the expected process steps and physical state, see Figure 1 below.

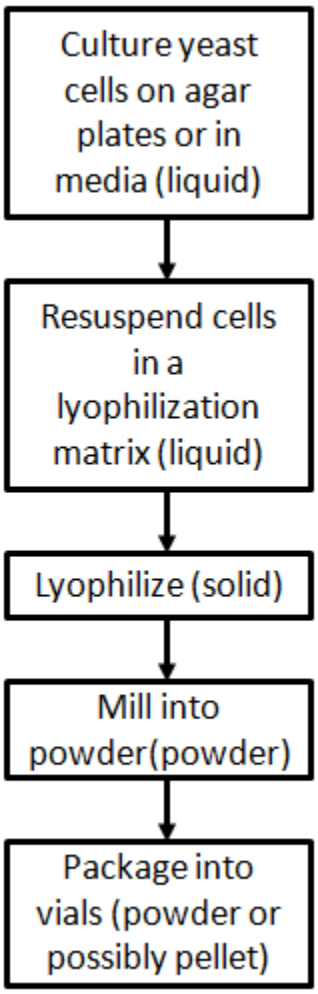

Figure 1. Lyophilization Process Flow Diagram

ENVIRONMENTAL RELEASE SUMMARY

WATER/AIR/INCINERATION/LANDFILL:

From: Handling Powdered Yeast

Amount: 1.5×10^9 CFU/day, 2 days/yr

Basis:

- 0.5% loss to water, air, incineration, or landfill (per EPA/OPPT Solids Transfer Dust Transfer Model)

- 6.0×10^{11} CFU/yr (calculated Total PV – see above)
- 2 days of production(see contact report)
- 90% inactivation efficiency (submitter estimate) for lyophilization and rehydration.

$= (6.0 \times 10^{11}\ \text{CFU/yr}) \times (0.005) \times (1-0.9)$

$= 3.0 \times 10^{8}\ \text{CFU/yr}$

$= 1.5 \times 10^{8}\ \text{CFU/day}$

OTHER WATER: Negligible

Basis: The technical contact indicates that laboratory waste will be autoclaved or treated with bleach, per standard laboratory procedures. CEB assumes that this small-scale inactivation is nearly 100% efficient and results in a negligible release of viable organisms (consistent with Biotech GS treatment of spent samples and past cases).

OTHER LANDFILL: Negligible

Basis: No sources of release to this medium have been identified other than potential releases from residue in equipment. The technical contact indicates that laboratory waste will be autoclaved or treated with bleach, per standard laboratory procedures. CEB's standard assumption for treatment of laboratory-scale equipment (<10 liters) is that releases are negligible (consistent with past cases).

OTHER INCINERATION: Not expected

Basis: No sources of release to this media have been identified (nor are they typically expected, per CEB generic scenario)

OCCUPATIONAL EXPOSURE

Number of Total Workers: 3

Basis: The technical contact was not sure of the number of workers potentially exposed to the MCAN. EPA assumes a minimum of 3 workers/site.

Days/yr: 2 (see above)

PPE: The submitter indicated that the workers will wear gloves, protective clothing, and eye protection (p. 25).

From: Laboratory activities - culture transfer, preparing the inoculums, and monitoring growth.

INHALATION:

(1) From: Laboratory Activities

Amount of Exposure:
3 workers, 2 days/year
<21.5 CFU/day

Basis:

Although air releases are expected to be negligible and inhalation exposures may be mitigated by use of standard aseptic techniques, CEB's biotech generic scenario recommends assuming inhalation exposures in a laboratory setting as a worst case. The recommended method for estimating potential inhalation exposures is to take the most applicable area monitoring data collected by NIOSH in a fermentation facility and multiply it by an estimate of the exposure duration. The NIOSH study listed the CFU concentration in a laboratory setting to be in the range from 32 to 103 CFU/m^3. Note that for recent GEM evaluations, the assumptions (and corresponding data) for exposures from 10 minutes of pipetting were used as analogous data to represent potential exposures to the microorganisms of this MCAN during laboratory operations.

- $[CFU]_{WA}$ = 32 to 103 CFU/m^3 (GS estimate for laboratory setting)
- I (inhalation rate)= 1.25 m^3/hr
- H = hours per day = 0.167(CEB assumption)
- 2 days/yr (see above)
- 3 workers (see above)

Calculation:

E_I = (I)(h)($[CFU]_{WA}$) (per GS)

= (1.25 m^3/hr)(0.167 hr/day)(32 to 103 CFU/m^3)

= 6.7 to 21.5 CFU/day

(2)From: Milling

Amount of Exposure:

3 workers/site, 2 days/yr
<4.8 X 10^3 CFU/day

Basis:

CEB assumes that potential inhalation exposure may occur during laboratory milling of the yeast. CEB uses the EPA Small Volume Handling Model to estimate exposures. Note that the particle sizes are approximately 100 microns, per technical contact.

- 0.0477 to 0.161 mg exposure/kg solid handled (EPA Small Volume Handling Model, less than 54 kg of solid containing the PMN handled per site-day)
- 6.0 x 10^{11} CFU/yr (calculated Total PV – see above)
- 1 x 10^9 CFU/g (per same submitter analog past case R15-0003)
- ~30 g produced per day [calc: (6.0 x 10^{11} CFU/yr)/ (2 days/yr) / (10^9 CFU/g) = 30 grams/day]
- 3 workers (see above)

Calculation:

=(0.0477 to 0.161 mg exposure/kg solid handled)(30 g handled)(kg/1000g) (1 x 10^9 CFU/g)(g/1000 mg)

= 1.4 X 10^3 to 4.8 X 10^3 CFU/day

DERMAL:

Amount of Exposure:
3 workers, 2 days
9 x 10^6 CFU/day

Basis:
Biotech generic scenario. The potential dermal dose rate is the product of CEB standard dermal exposure assessment factors and the CFU concentration in the appropriate process stream. For bench-scale handling of liquids, the CEB standard dermal factor is up to 1,100 mg/day (per RAD's 2013 Updated Method for Screening-Level Estimates of Dermal Exposure). This factor can be used with the concentration of the GEM to estimate the dermal exposure (consistent with laboratory exposures for past cases).

- C = typical contact volume: <1,100 mg/day
- 1 x 10^9 CFU/g (per same submitter analog past case R15-0003)
- 2 days (see above)
- 3 workers (see above)

Calculation:

$E_D = ([CFU]_P)C$ (per GS)

= (<1,100 mg/day)(1 x 10^9 CFU/g)/(1000 mg/g)

= 1.1 x 10^9 CFU/day

INITIAL REVIEW ENGINEERING REPORT [redacted]

MCAN: J18-0044

USE: Biological Detection Workflows

Number of Sites/Locations: up to 48 sites

Unknown sites includes fire stations, academic facilities, companies. The technical contact did not have an estimate for number of sites (per technical contact). However, the submission states that 1-4 vials may be used for 1 training and 5×10^7 cells are in each vial (both live and dead). Therefore RAD calculates the number of sites using the following formula:

$= (6.0 \times 10^{11}$ CFU/yr)/[(1 to 4 vials/training)$(5 \times 10^7$ CFU/vial)]

= 3,000 to 12,000 trainings/yr

= (3,000 to 12,000 trainings/yr)(1 day/ training)/(250 days/yr)

= 12 to 48 sites

Days/yr: 250 days/year

Basis: The technical contact was not sure of the number of operating days. RAD assumes conservative 250 days/yr.

PROCESS DESCRIPTION:

The submission and technical contact indicated that the MCAN will be used to for training personnel on biological detection of biothreat agents. For example, the GEM can be crushed into a powder and used in lieu of a suspicious material to safely challenge the biological assessment workflow. In addition, the MCAN may be used to validate microbial enumeration technologies (e.g., flow cytometer) (p. 23-24 and technical contact). To be conservative, RAD assesses 100% release of the MCAN for the training use.

ENVIRONMENTAL RELEASE SUMMARY

Submission does not estimate release amounts but provides some information about water, air, and solid waste releases. Technical contact stated that all waste is heat inactivated prior to disposal with either an autoclave or bleach. In addition, the lyophilization and rehydration will decrease the viability of the CFUs to less than 10% (p. 23). RAD used the GS methodology to estimate the releases from the downstream uses. RAD based releases on the trainings (estimated to be 70% of the PV) since the releases and exposures are more conservative than the biological instrument application (~30% of PV)

WATER/AIR/INCINERATION/LANDFILL

1) From: Training Release

Amount: 5.0×10^6 to 2.0×10^7 CFU/site-day CFU/site-day over 250 days/yr for 48 sites

Basis: CEB assumes a 100% release scenario:

- 5.0×10^7 to 2.0×10^8 CFU/training. Submitter estimates that 1 to 4 vials are used for each training and each vial contains approximately 5×10^7 CFUs. Therefore, the following calculates the amount of CFUs per training:

  = (1 to 4 vials)(5×10^7 CFU/vial)

  = 5.0×10^7 to 2.0×10^8 CFU/training

- 90% inactivation efficiency (submitter estimate) for lyophilization and rehydration.

= (5.0×10^7 to 2.0×10^8 CFU/training)(1-0.9)

= 5.0×10^6 to 2.0×10^7 CFU/site-day

OCCUPATIONAL EXPOSURE

Number of Workers: up to 144 workers, 48 sites

Days/site-yr: 250 (see above)

Basis: The technical contact was unsure how many workers were exposed during the downstream uses. RAD assumes default minimum of 3 workers/site.

PPE: All personnel will be wearing appropriate personal protective equipment (PPE) for BL Safety level one material (per technical contact).

INHALATION:

From: Training Release

Amount of Exposure:

144 workers 250 days/yr
<483 CFU/day

Basis:

CEB assumes that potential inhalation exposure may occur during the trainings. The GEM will be crushed into a powder and handled by trainees. CEB uses the EPA Small Volume Handling Model to estimate exposures.

- 0.0477 to 0.161 mg exposure/kg solid handled (EPA Small Volume Handling Model, less than 54 kg of solid containing the PMN handled per site-day)
- 6.0×10^{11} CFU/yr (calculated Total PV – see above)
- 1×10^{9} CFU/g (per same submitter analog past case R15-0003)
- ~30 g produced per day [calc: $(6.0 \times 10^{11}$ CFU/yr)/ (2 days/yr) / $(10^{9}$ CFU/g) = 30 grams/day]
- 90% inactivation efficiency (submitter estimate) for lyophilization and rehydration.
- 3 workers/site (see above)

Calculation:

=(0.0477 to 0.161 mg exposure/kg solid handled)(30 g handled/day)(kg/1000g) (1 x 10^9 CFU/g)(g/1000 mg)(1-0.9)

= 143 to 483 CFU/day

DERMAL:

From: Training Activities

Amount of Exposure:

144 workers, 250 days/yr
3.1 X 10^8 CFU/day

Basis:

Biotech GS - The potential dermal dose rate is the product of CEB standard dermal exposure assessment factors and the weight fraction of CFU in the appropriate process stream. CEB assumes that dermal exposure occurs during training.

- 3,100 mg/day (routine, direct 2-hand handling of solids, CEB standard model)
- 1 x 10^9 CFU/g (per technical contact)
- 144 workers (see above)
- 90% inactivation efficiency (submitter estimate) for lyophilization and rehydration.

Calculation:

(3,100 mg/day)(1 x 10^9 CFU/g)(g/1000 mg)(1-0.9)

= 3.1 X 10^8 CFU/day

[redacted] **CONTACT REPORT** **(MCAN: J18-0044)**

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Submitter: National Institute of Standards and Technology (NIST)
DATE: 9/5/2018

Person Contacted: Dr. Nancy J. Lin
Affiliation: Acting Leader, Biomaterials Group, NIST

Telephone: (301) 975-4935

Caller: Anna Dimling
Affiliation: ERG for RAD

1. **Do you know if this microorganism produces spores?**

   No this GEM does not produce spores.

2. **You indicate that the microorganism is subject to autoclaving, can you provide the inactivation efficiency? Can you confirm that all waste is autoclaved?**

   All the waste would be autoclaved or bleach. Not sure what the media of release would be. The submitter is not sure of the inactivation efficiency for the autoclaving and sodium hypochlorite treatment and does not have cell kill data.

3. **How many days/yr per batch for first year and third year fermentation?**

   The submitter thinks it would be a couple days. Another company does the fermentation, but not sure what company will win the bid yet.

4. **Would this process also take place in a laboratory setting (autoclave wastes, process under hood ventilation, etc.)**

   The submitter is not sure how the company will perform the fermentation process. They still have to put the contract out to bid to a contract site for the manufacturing of the GEM. Only then will the submitter know the site's practices. However, the technical contact said this site would likely be Microbiologics, Inc. in St. Cloud, MN (consistent with R15-0003).

5. **What are more specifics about the use?**
    - 30% of USE - Mostly be in the government or academia for the instruments (e.g.,flow cytometer), and these facilities should have the appropriate decontamination protocol. The product is labeled as BL Safety level one and waste needs to be treated appropriately by the use sites.
    - 70% of USE - The GEM is designed for field tests in laboratory or fire station. The personnel are trained to collect biohazard waste. Have a robust protocol for dealing with hazardous chemicals and waste.